09/914874

JC03 Rec'd PCT/PTO 05 SEP 2001

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Docket No.: **F-7129**
Filing Date: **September 5, 2001**

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[ ] ATTN: BOX PATENT APPLICATION

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[x] THIS IS THE 35 U.S.C 371 NATIONAL STAGE OF **PCT/EP00/01701** FILED **February 25, 2000**

Sir:

Transmitted herewith for filing is the [x] Utility [ ] Design nonprovisional patent application of:

Inventor / Application Identifier: **Johann Michael KOEHLER**

[ ] See Inventor Information Sheet attached

For: **MICROCOLUMN REACTOR**

[ ] This is a new patent application.
[x] This is the 35 U.S.C. 371 National Stage Application of the above-identified PCT Application.
[ ] This is a: [ ] Continuation Application
[ ] Divisional Application
[ ] Continuation-in-Part Application
of prior Application Serial No. _.

[ ] Cancel in this application original claims ___ of the prior application before calculating the filing fee.

[ ] Amend the specification by inserting before the first line the sentence:
-- This is a [ ] Continuation, [ ] Division, [ ] Continuation-in-part, of Application

[ ] Incorporation By Reference. The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

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ENCLOSED ARE THE FOLLOWING:		
X	6	Sheets of drawings ([x] formal [ ] informal size A4).
X	20	Pages of specification including abstract and claims.
X	26	Total pages.
X	Combined Declaration and Power of Attorney	
	X	Newly executed
		Copy from prior application
		Inventors deleted; see attached statement
	Sequence Listing	
		Computer Readable Copy
		Paper copy
		Statement verifying identity of above copies
X	Return Receipt Postcard	
X	Preliminary Amendment	
	Assignment to:	
		Assignment is of record in prior application Serial No._.
		Assignment Recordation Form Cover Sheet.
		Charge $40.00 to Deposit Account No. 10-1250 for recording Assignment.
X	Information Disclosure Statement	
X	Information Disclosure Citation	
	English translation	
X	Application Data Sheet	

PRIORITY CLAIMS		
	Applicant hereby claims the benefit of the filing date of the following provisional application(s) under the provision of 35 USC 119.	
X	Applicant hereby claims the benefit under the provisions of 35 USC 119 of the filing dates of the following applications as indicated below: **Germany Patent Appln. No. 199 10 392.5, filed March 5, 1999, Priority Claimed** of which certified copies thereof	
		will follow
		are enclosed
	X	have been filed in the International Bureau
		were filed in prior application:

CLAIMS FILED AND FILING FEE CALCULATION					
ITEM	—			Rate	Applied Fee
[ ] Base Fee - Non PCT	—			$710	
[ ] Base Fee - PCT IPEA-US	—			$690	
[ ] Base Fee - PCT ISA-US	—			$710	
[ ] Base Fee - PCT not ISA or IPEA	—			$1,000	
[X] Base Fee - PCT with EPO or JPO Search Report	—			$860	$860
[ ] Base Fee - Design	—			$320	
Claim Fees	Number Filed	Base Number	Number Extra over Base	—	
Total Claims	30	20	10	$18	$180
Independent Claims	1	3	0	$80	$0
Multiple Dependent Claim Fee	—			$270	$0
[ ] Small Entity Status is Asserted	—				($0)
[ ] Foreign Language Filing Fee	—			$130	$0
TOTAL FILING FEE					**$1,040**

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JORDAN AND HAMBURG LLP

By

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09/914874

JC03 Rec'd PCT/PTO 05 SEP 2001

F-7129

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Johann Michael KOEHLER
Serial No.	:	Not yet known (U.S. National Stage of PCT/EP00/01701 filed February 25, 2000)
Filed	:	Concurrently herewith
For	:	MICROCOLUMN REACTOR

Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Preliminary to examination, please amend this application as follows:

IN THE CLAIMS:

Amend claims 1-15 as follows:

1. (Amended) Microcolumn reactor for carrying out reactions on solid phases and/or biological cells comprising at least a first and a second substrate wafer being engaged to one another in a common plane, whereby at least one longitudinally extending channel is inserted into at least one of said substrate wafers, said channel, in a preselectable section of its length, being captured by two passage openings, which are passed through the substrate wafer, whereby filter elements are provided, and wherein the passage openings are separated from the

channel by a partially permeable sieve-like membrane, the membrane having transmission areas so dimensioned that they preselectably prevent micro-beads and/or cells, which are introduced into the channel, from entering into the passage openings, and the channel is provided with at least two further openings outside of the section captured by said passage openings, said at least two further openings being adapted to enable a loading and/or a displacement of the micro-beads and/or cells, provided above the section captured by said passage, by applying a fluidic pressure, and further comprising means for temporarily closing at least one of the passage openings and one of the further openings.

2. (Amended) A microcolumn reactor as claimed in claim 1, wherein glass is selected for the first substrate wafer and a silicon wafer for the second substrate wafer, whereby the channel is inserted into the glass plate and the surface of the silicon wafer opposing said glass plate is entirely covered by a coat, into which a micro-structurized perforation is provided at least in the section of the passage openings, said micro-structurized perforation being for forming transmission areas.

3. (Amended) A microcolumn reactor as claimed in claim 1, wherein at least one of a glass plate and a plate made of synthetic material is selected for the first and/or for the second substrate wafer, the channel is inserted into the first substrate wafer and the surface of the second substrate wafer opposing said first

substrate wafer is entirely covered by a membrane, into which a micro-structurized perforation is provided at least in the section of the passage openings, said micro-structurized perforation being for forming transmission areas.

4. (Amended) A microcolumn reactor as claimed in claim 3, wherein the membrane is a perforated polymeric foil.

5. (Amended) A microcolumn reactor as claimed in claim 2, wherein the first and the second substrate wafer are connected to one another by anodic bonding.

6. (Amended) A microcolumn reactor as claimed in claim 2 or 3, wherein the first and the second substrate wafer are connected to one another by adhesives outside of the channel.

7. (Amended) A microcolumn reactor as claimed in claim 3, wherein the first and the second substrate wafer are connected to one another by externally provided clamping means.

8. (Amended) A microcolumn reactor as claimed in claims 1, 2 or 3, wherein the passage openings are connected to a respective additional channel

which is arranged in the plane of the second substrate wafer and extends to a rim of the substrate.

9. (Amended) A microcolumn reactor as claimed in one of claims 1 to 5 and 7, wherein the channel is defined by a plurality of passage openings, and respectively correlated passage openings, which constitute an inlet and an outlet, and passage openings, which define a section of the channel, are arranged to one another equidistantly or in variable and different distances.

10. (Amended) A microcolumn reactor as claimed in claim 9, wherein a plurality of the correlated passage openings are provided on a common substrate or a plurality of discrete microcolumn reactors are fluidically interconnected, the respective distances of correlated passage openings, which form one inlet and one outlet, being formed, adapted to an actual reaction process, of different length.

11. (Amended) A microcolumn reactor as claimed in claim 1, wherein a plurality of substrate wafers, which have a respective said channel and respective said two passage openings each, are linearly and/or in a plurality of planes fluidically interconnected with one another as separate units each, and further components are provided at preselectable connection sites.

12. (Amended) A microcolumn reactor as claimed in claim 1, wherein a plurality of substrate wafers, which have a respective said channel and respective said two passage openings each, are linearly and/or in a plurality of planes fluidically interconnected with one another as separate units each, and further micro-structurized components are provided integrated in the entire system.

13. (Amended) A microcolumn reactor as claimed in claim 2, wherein the passage openings are, in parallel to the surface normal, formed by two channel sections in the shape of two truncated pyramids standing via their small base faces top-to-top one upon the other, an Si(100)-wafer is used for the second substrate wafer which on both of its sides is provided with an etching mask, a first masking face of which is provided with transmission areas at least across the passage openings, and the second masking face disposed on the opposing wafer face is provided with recesses, the openings of which correspond to the smallest inside cross section of the passage openings.

14. (Amended) A microcolumn reactor as claimed in claim 8, wherein the second substrate wafer is a Si-wafer of 100-orientation or 110-orientation, which on one of its sides is provided with a sieve pore membrane mask structure which, in the vicinity of the additional channel is accompanied by a window corresponding

to the channel width, which extends up to a rim of the chip, and the opposite side of the Si-wafer is entirely covered by a protective coat which is etching resistant.

15. (Amended) A microcolumn reactor as claimed in claim 1, wherein the membrane is a nano-porous thin-layer membrane, the pore sizes of which are in a range of 5 to 500 nm.

IN THE ABSTRACT:

Delete the original abstract and substitute therefor the abstract appended hereto on a separate sheet.

ABSTRACT

A microcolumn reactor includes at least one first and one second substrate wafer linked together across their surfaces. At least one of the substrate wafers has an elongate channel comprising in a defined section of its length two passage openings that are implemented in the opposite substrate wafer. The openings are separated from the channel by a partially permeable sieve-type membrane. The areas of acceptance of the membrane are of a diameter specifically preventing microbeads and/or cells introduced into the channel from entering the passage openings. The channel has at least two further openings outside the section that is comprised by the passage openings. The further openings permit the microbeads and/or cells that are provided above the section to be introduced and/or displaced by applying a fluid pressure. Means are provided that temporarily close at least one of the passage openings and one of the further openings.

REMARKS

The claims have been amended only formally and the abstract has been shortened to the required not more than 150 words.

Respectfully submitted,

JORDAN AND HAMBURG LLP

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APPENDIX I

1. (Amended) Microcolumn reactor for carrying out reactions on solid phases and/or biological cells comprising at least a first and a second substrate wafer [(l; 2)] being engaged to one another in a common plane, whereby at least one longitudinally extending channel [(3)] is inserted into at least one of said substrate wafers [(1; 2)], said channel, in a preselectable section [(a)] of its length, being captured by two passage openings [(41, 42)], which are passed through the substrate wafer, whereby filter elements are provided, [characterized in that] and wherein the passage openings [(41, 42)] are separated from the channel [(3)] by a partially permeable sieve-like membrane [(5; 50)], the membrane having transmission areas [(51) of said membrane are] so dimensioned that they preselectably prevent micro-beads and/or cells [(7)], which are introduced into the channel [(3)], from entering into the passage openings [(41, 42)], and [in that] the channel [(3)] is provided with at least two further openings [(61, 62)] outside of the section [(a)] captured by said passage openings [(41, 42)], said at least two further openings [(61, 62)] being adapted to enable a loading and/or a displacement of the micro-beads and/or cells [(7)], provided above the section [(a)] captured by said passage, by applying a fluidic pressure [(p)], and [in that] further comprising means [(91, 92) are provided] for temporarily closing [of] at least one of the passage openings [(41, 42)] and [of] one of the further openings [(61, 62)].

2. (Amended) A microcolumn reactor as claimed in claim 1, [characterized in that] wherein glass is selected for the first substrate wafer [(1)] and a silicon wafer for the second substrate wafer [(2)], whereby the channel [(3)] is inserted into the glass plate [(1)] and the surface of the silicon wafer [(2)] opposing said glass plate is entirely covered by a coat, into which a micro-structurized perforation is provided at least in the section of the passage openings [(41, 42)], said micro-structurized perforation being for forming transmission areas [(51)].

3. (Amended) A microcolumn reactor as claimed in claim 1, [characterized in that] wherein at least one of a glass plate [and/or] and a plate made of synthetic material [is/are] is selected for the first and/or for the second substrate wafer [(1, 2)], [whereby] the channel [(3)] is inserted into the first substrate wafer [(1)] and the surface of the second substrate wafer [(2)] opposing said first substrate wafer [(1)] is entirely covered by a membrane, into which a micro-structurized perforation is provided at least in the section of the passage openings [(41, 42)], said micro-structurized perforation being for forming transmission areas [(51)].

4. (Amended) A microcolumn reactor as claimed in claim 3, [characterized in that] wherein the membrane is [formed by] a perforated polymeric foil [(50) covering the second substrate wafer (2)].

5. (Amended) A microcolumn reactor as claimed in claim 2, [characterized in that] <u>wherein</u> the first and the second substrate wafer [(1, 2)] are connected to one another by anodic bonding.

6. (Amended) A microcolumn reactor as claimed in claim 2 or 3, [characterized in that] <u>wherein</u> the first and the second substrate wafer [(l, 2)] are connected to one another by adhesives outside of [the range of] the channel [(3)].

7. (Amended) A microcolumn reactor as claimed in claim 3, [characterized in that] <u>wherein</u> the first and the second substrate wafer [(1, 2)] are connected to one another by externally provided clamping means.

8. (Amended) A microcolumn reactor as claimed in claims 1, 2 or 3, [characterized in that] <u>wherein</u> the passage openings [(41, 42)] are connected to a respective <u>additional</u> channel [(411, 421)] which is arranged in the plane of the second substrate wafer [(2)] and extends to [the] <u>a</u> rim [portion] of the substrate.

9. (Amended) A microcolumn reactor as claimed in one of [the preceding] claims <u>1 to 5 and 7</u>, [characterized in that a] <u>wherein the</u> channel [(3)] is defined by a plurality of passage openings [(41, 42)], [whereby] <u>and</u> respectively correlated passage openings, which constitute an inlet [(i)] and an outlet [(o)], and passage

openings, which define a section [(a)] of the channel [(3)], are arranged to one another equidistantly [(b)] or in variable and different distances [(c)].

10. (Amended) A microcolumn reactor as claimed in claim 9, [characterized in that,] wherein [when] a plurality of the correlated passage openings [(41, 42)] are provided on a common substrate or [when] a plurality of discrete microcolumn reactors are fluidically interconnected, the respective distances [(a; a1)] of correlated passage openings, which form one inlet and one outlet, [are designed] being formed, adapted to [the] an actual reaction process, of different length.

11. (Amended) A microcolumn reactor as claimed in claim 1, [characterized in that] wherein a plurality of substrate wafers, which have a respective said channel [(3)] and respective said two passage openings [(41, 42)] each, are linearly and/or in a plurality of planes fluidically interconnected with one another as separate units each, [whereby] and further components [(8) such as, for example, optical detectors, analysis units, calorimeters, electrochemical detectors etc.] are provided at preselectable connection sites.

12. (Amended) A microcolumn reactor as claimed in claim 1, [characterized in that] wherein a plurality of substrate wafers, which have a

respective said channel [(3)] and respective said two passage openings [(41, 42)] each, are linearly and/or in a plurality of planes fluidically interconnected with one another as separate units each, [whereby] and further micro-structurized components [(8) such as, for example, optical detectors, analysis units, calorimeters, electrochemical detectors etc.] are provided integrated in the entire system.

13. (Amended) A microcolumn reactor as claimed in [claims 1 and] claim 2, [characterized in that] wherein the passage openings [(41, 42)] are, in parallel to the surface normal [n], [designed] formed by two channel [ranges] sections in the shape of two truncated pyramids standing via their small base faces top-to-top one upon the other, [whereby] an Si(100)-wafer is used for the second substrate wafer [2] which on both of its sides is provided with an etching mask, [the] a first masking face of which is provided with transmission areas [(51)] at least across the passage openings [(41, 42)], and [whereby] the second masking face disposed on the opposing wafer face is provided with recesses, the openings of which correspond to the smallest inside cross section of the passage openings [(41, 42)].

14. (Amended) A microcolumn reactor as claimed in [claims 1, 2, and] claim 8, [characterized in that an] wherein the second substrate wafer is a Si-wafer of 100-orientation or 110-orientation [is used for the second substrate wafer],

which on one of its sides is provided with a sieve pore membrane mask structure [(5)] which, in the [range] vicinity of the [further extending] additional channel [411, 412,] is accompanied by a window corresponding to the channel width, which extends up to [the] a rim of the chip, and the opposite side of the Si-wafer is entirely covered by a protective coat[, for example Si_3N_4,] which is etching resistant.

15. (Amended) A microcolumn reactor as claimed in claim 1, [characterized in that] wherein the membrane [(5)] is [formed by] a nano-porous thin-layer membrane, the pore sizes of which are [defined] in a range [between 5…500] of 5 to 500 nm.

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MICROCOLUMN REACTOR

BACKGROUND OF THE INVENTION

The invention relates to a microcolumn reactor for carrying out reactions on solid phases and/or with biological cells. Thereby, the proposed microcolumn reactor can be applied, for example, with advantage in the following synthesis processes and separation processes:

- in Grignard reactions and in the manufacture of other metal-organic compounds,
- in drying of solvents by aid of metallic or oxidic dessicants,
- in ion exchange processing, and
- in the extraction of solid phases.

More particularly, the invention will find application in the field of complex combinatorial-chemical operations or in screening operations or in automatically carrying out biological screening processing.

Sample particles ("beads" or "Perlen") have been used in separations and synthesis in the laboratory work for decades. These particles mostly are glass or polymeric globules that have diameters of 0.01 mm up to 1 mm, typically about 0.1 mm, which are filled, dry or pre-swelled, as a loose material into a receptacle where they are then flushed by a liquid, whereby an adsorption process or a reaction process takes place between the solid phase surface of the particles and the liquid surrounding the particles. Methods of the column chromatography (for example, gel filtration), of the column extraction, of the immundiagnosis, of the bio-

molecule purification (for example, DNA cleaning), as well as of the homogeneous and heterogeneous synthesis (for example, of oligonucleotides, peptides or combinatorial substance libraries) utilize these techniques. With all these methods and devices, a solid-phase is stationarily integrated during the reaction in the reaction vessel, whereby reactants are bound to the solid phase or the latter serves as a reaction surface. The processes comprise a sequence of individual binding operations and separation operations. In such devices, a removal of the stationarily arranged solid phase from the reactor in the course of an operation series is either not provided for, or not possible or it is time consuming and not very practical.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a microcolumn reactor for synthesis and separation processes on small sample volumes, by aid of said microcolumn reactor, instead of an alternating sequence of binding and elution processes of a solid phase that is stationarily bound during an operation series, an exchange of the stationary solid phase, for example, after loading can be performed in order to more efficiently separate, for example, complex substance mixtures or particles and cells and to carry out combinatorial syntheses more easily and faster.

The object is realized by the features of the first claim. Advantageous embodiments are covered by the dependent claims.

The very essence of the invention consists in that a microcolumn reactor for carrying out reactions on solid phases and/or biological cells is provided, which is comprised of at least a first and a second substrate wafer which are linked with one another in a common plane and whereby at least one longitudinally extending channel is provided in at least one of the substrate wafers, said channel, in a preselectable section of its longitudinal extension, is captured by two passage openings which are provided in and into the opposite substrate wafer. The passage openings are separated from the channel by a partially permeable sieve-like membrane, the transmission range of which is so dimensioned as to preselectably prevent the microbeads and/or cells, which are introduced into the channel, from entering the passage openings. The channel is provided with at least two further openings which are disposed outside the section defined by the passage openings, said further openings are adapted to introduce and/or to displace the microbeads and/or the cells provided in, respectively above the section by applying a fluid pressure. At least one of the passage openings and one of the further openings are adapted to be closed.

DETAILED DESCRIPTION OF THE INVENTION

The invention will be explained in more detail by virtue of schematical embodiments. There is shown in:

Fig. 1 an exploded view of a first embodiment of a microcolumn reactor with its functionally essential components,

Fig. 2 an exploded view of a second embodiment of a microcolumn reactor with its functionally essential components,

Fig 2a another type of connection of the passage openings with inlet channels and outlet channels, respectively,

Fig. 3 a longitudinal section through a part of the embodiment according to Fig. 1 or Fig. 2,

Fig. 4 and 5 possible arrangement designs of a plurality of microcolumn reactors,

Fig. 6 a block diagram exemplifying the connection of a plurality of microcolumn reactors for the synthesis of a special substance library, and

Fig. 7a-d a further embodiment of a microcolumn reactor in different steps of manufacture.

In Fig. 1, number 1 designates of a first embodiment of a microcolumn reactor shown in an exploded view with its functionally essential components. According to Fig. 1, the microcolumn reactor is comprised of two substrate wafers 1, 2, having, in the present example, an elongated channel 3 inserted into said first substrate wafer 1 which here is made of glass and has the dimensions of 15 mm · 8 mm · 1 mm. The channel 3 is given a width of 1 mm, a depth of 100 µm and a length of 10 mm. Said channel is connected via two openings 61, 62, which here are bore holes, to two fluid connection ports (not shown). As to the second substrate wafer 2, a silicon chip having the dimensions of 15mm·8mm·0.5mm has been selected, into which two passage openings 41, 42 have been worked

in in such a manner that, when precisely position fitted relative to the glass plate 1, they capture a section a, which in the example comes to lie across the end portions of the channel 3. The passage openings 41, 42 are covered by a partially permeable membrane of the sieve type, here in the form of a porous membrane of siliconoxinitride. In the example, the membrane has a thickness of 2μm and the transmission areas 51 of the membrane have pores with diameters of 5μm. Depending on the special case of application and on the microbeads 6 to be used, the actual sizes of the transmission area 51 can be variably and preselectably designed. The pore membrane has the task to hold back the particles or cells 7 that are to be introduced into the channel 3. Furthermore and depending on the kind of application of the microcolumn reactor, the membrane 5 can, within the frame of the invention, be formed by a nano-porous thin layer membrane, the pore size of which can be selected to lie within a range between 5...500 nm.

If there is intended to provide to the passage openings 41, 42 a shape as indicated in Fig. 3, where the passage openings, being in parallel to the surface normal n of the second substrate wafer 2, are formed by two channel ranges in the shape of two truncated pyramids standing via their small base faces top-to-top, then a Si(100)-wafer is used for the substrate wafer 2 which on both of its faces is provided with a suitable etching mask (for example, Si-oxinitride). Said mask, on the front side, forms an array of micro-windows, the dimensions of which define the pore width. Typically, a pore width of 5 μm is realized. This can easily be manufactured by conventional means of the photolithography and the

etching technique. Two windows are structurized from the rear side of the Si-wafer centrally to this pore structure in such a way that two channel ranges in the shape of two truncated pyramids result from the etching procedure carried out on both sides, whereby the surfaces of said channels which are in opposition to the first substrate wafer 1 are closed by sieve bottoms.

When at the same time with generating the passage openings 41, 42 channel structures adapted for a fluid contact of the mobile phase within the substrate plane of the substrate wafer 2 have to be generated, so that the connection ports 41, 42 are, for example, laterally funneled out from the leading faces of the microcolumn reactor, then in the present example, there is a Si-wafer (in a 100-orientation for inclined channel walls or in a 110-orientation for vertical channel walls) employed for the substrate wafer, which is provided with a sieve pore membrane mask structure 5 which, in the range of the extending channel 411, 412, is accompanied by a window which corresponds to the channel width and which extends up to the rim of the chip (refer to Fig. 7a-d). The rear side of the Si-wafer is entirely covered by a protective coat (for example Si_3N_4) which is resistant to etching. At first, the Si-wafer is etched in an etching bath which acts isotropically (for example, on HF-basis) in such a way that the stems between the individual pores of the sieve structure mask are completely underetched. Subsequently the etching is continued in an anisotropic etching bath (for example, on a KOH-basis), to obtain the inclined and vertical, respectively, edges of the channel. In Fig. 7a-d the results of the foregoing procedure is shown by means of an exemplary

structure. Thereby in Fig. 7a a channel 3 can be seen which is inserted into the first substrate wafer 1. Fig. 7b shows the structure inserted into the first substrate wafer 1, including the formed channel sections 411 and 421 extending up to the rim portion of the substrate, whereby Fig. 7c shows the relations represented in Fig. 7b in a lateral section, and Fig. 7d illustrates in a transparent plan view the orientation of the individual parts mentioned relative to one another with the first substrate wafer and the second substrate wafer being connected to one another.
The first and the second substrate wafers 1,2 described in connection with Fig. 1 are connected with one another by anodic bonding after manufacture of the structures described.

The special embodiments described hereinbefore are particularly advantageously to be manufactured by conventional working steps of approved microstructuring techniques, however, the invention is not limited thereto. It is possible as well, to use a glass-wafer and/or a wafer made of a synthetic material for the first and/or the second substrate wafers 1,2, whereby the channel 3 is inserted into the first substrate wafer 1 and the surface of the second substrate wafer 2 opposing said first substrate wafer 1 is entirely covered by a membrane in which at least in the range of the passage openings 41, 42 a microstructurized perforation is provided in order to form the transmission areas 51. In particular, here the second substrate wafer 2 can be provided with a perforated polymeric foil 50 covering the substrate wafer, as schematically indicated in Fig. 2. Such an embodiment even allows for other pathways for the channel, as

indicated in Fig. 2a by way of example with respect to the channel pathways 411 and 421 in the substrate wafer 2.

In Fig. 3 there is shown a longitudinal section through a part of the embodiments according to Fig. 1 and 2 which have already been described. Thereby it is rendered visible how the channel 3 with the substrate wafers 1, 2 in their assembled state is filled with the solid phase, here in the form of microbeads 7. With the present proposal it is not necessary to have extreme packing densities of the stationary phase 7. It is only important that the distances between the particles of the stationary phase 7 are so dimensioned that a molecule contacts the stationary phase frequently enough during its dwell time in the reactor chamber, which is here to be understood as the section of the channel 3 between the passage openings 41, 42. This will already be given with particle sizes in the mean to the lower micrometer range at hold-up times of less than one second, partially in the lower millisecond range. The definition of the particle sizes and/or of the cells to be retained or passed through depends on the given actual task and on the pore sizes, which have to be adapted accordingly, of the partially permeable sieve-like membrane 5. The pore size has to be so dimensioned that any blocking of the pores is eliminated. In Fig. 3 there is also schematically indicated that the individual flow paths in the microcolumn reactor shown are selectively closeable by at least two valves 91, 92. The openings 42, 62 can each optionally be closed by a respective further valve, not shown in Fig. 3. This permits a freely selective addressing of the two inlets 61, 41 and of the two outlets

42, 62 of the microcolumn reactor. Thus a microcolumn reactor is provided in which not only the mobile phase, for example, a homogeneous fluid, the flow path of which is indicated in Fig. 3 by the arrows i and o, can be moved but also the stationary phase, here the microbeads 7. The closing of one respective inlet flow or outlet flow now permits to selectively transport the mobile phase and/or the stationary phase 7 as a suspension. Thus a fast exchange of separation material will be possible, and there can be even built up solid phase separation logics and synthesis logics by a combination of a plurality of the microcolumn reactors described. Due to the micro-fluidic separation of the mobile phase and the stationary phase, specifical binding processes and separation processes can thus be carried out, which will be described in more detail in the following.

In Fig. 4 and 5 there are schematically indicated several possible forms of arrangement of a plurality of microcolumn reactors. Thereby, depending on the kind of realization, the microcolumn reactors can be, in analogy to Fig. 1 and 2, of a discrete design and can be combined with one another. Alternatively, as shown in Fig. 4 and 5, a plurality of such microcolumn reactors can be in a linear combination (Fig. 4) of a first and a second substrate wafer each, or there can be connections between a plurality of such microcolumn reactors in different planes (Fig. 5), whereby one respective plane is designed in analogy to Fig, 4. Depending on the preselectable reaction procedure, the proper reactor sections, defined by the length of section a, whereby the passage openings 41, 42 determine the channel 3 length, can be arranged to one another equidistantly by a

distance b or in a variably selectable distance c. It is also possible to select different lengths for the distances of the passage openings 41, 42 from one reactor to the next, refer to distance a and a1 in Fig. 4. The mentioned feasibilities are only determined by the reactions to be carried out with the microcolumn reactors. Further components, in particular such also designed in microsystem techniques, as for example, optical detectors, analysis units, calorimeters, electrochemical detectors etc., can be comprised in an interconnection of a plurality of microcolumn reactors, as indicated, only schematically, by such a unit 8 in Fig. 5.

The proposed microcolumn reactor and its multifold application by an interconnection of a plurality of individual microcolumn reactors is particularly suited for performing automated processes of agent development by means of bead-bound solid phase synthesis. A plurality of such microcolumn reactors can be connected to one another and by way of valves, which have to be provided, in order to obtain fluid-logics that permit to carry out, for example, more complex combinatorial-chemical or screening operations in a micro-automated way. Particularly in biologic screening processes, also cells can be introduced into the system instead of the micro-beads, indicated in Fig. 3. The reactor is also suited for the micro-modular combination with micro-flow cuvettes in micro-photometric, micro-fluorimetric, or micro-chemo-luminometric measurements.

The proposed microcolumn reactor permits a re-charging by pushing new reactants (micro-beads and/or cells) through the channel 3 in that a fluid

pressure p is applied so that a further variability is given for the entire device at a simultaneously lowest possible dead volume.

The represented advantageous possibilities of application will be indicated in more detail by way of the following examples.

At first, the synthesis of a library of 4 tripeptide gly-val-leu, gly-gly-leu, gly-val-ala, and gly-gly-ala will be described in a micro-reactor fluid system.

In the example, a system of 16 microcolumn reactors will be employed, which are fluidically interconnected, as indicated in Fig. 6. At first, the micro-reactors A1 and B1 as well as C1 and D1 are charged with micro-beads in the form of polystyrene-synthesis-beads, to the surfaces of which the amino acid glycine is coupled by an ester linkage to a benzyl group as a spacer. After blocking the fluid channels for the stationary phases (beads) via the channels, the inlets and outlets of which are provided with sieve bottoms, the four microcolumn reactors are flushed for the mobile phases in pairs (A/B) by a 1:1 mixture of a warm solution of dicyclohexylcarbodiimide and valine, respectively (C/D) by a 1:1 mixture of warm solution of dicyclohexylcarbodiimide and glycine. Thereby the amino groups of the added amino acids are protected by tertiary-butyloxycarbonyl-groups. After the first flushing step, all the stationary phases are moved on to the module row 2 by a respectively applied fluidic pressure, and are flushed there by the series-connected channels to be ready for the mobile phase and are de-protected by passing through a slightly aqueous trifluoroacetic acid. Subsequently, the fluid phases are

moved on to the module group 3, whereby the stationary phase of B2 is moved on to C3 and that of C2 on to B3. In the module row 3, there is carried out, in analogy to the module row 1, the transfer by protected amino acids, whereby leucine is now added instead of valine, and alanine is now added instead of glycine. After this operation and after a first elution step, the stationary phases are moved on to the module row 4 and there the protective groups are separated. Subsequently the four groups of synthesis-beads are then removed at separate outlets and, in order to release the tripeptides, the benzylester links are separated.

In a further example for application the manufacture of ethyl-grignard from ethylchloride for micro-fluid-syntheses will be described.
At first, as a stationary phase, a suspension of magnesium powder in dry ethylether is loaded into a microcolumn reactor. After closing the channel for this stationary phase, and to make ready for the mobile phase, ether is displaced by a warm solution of ethylchloride in ether via the inlet and the outlet. At the outlet of the mobile phase ethyl-grignard is taken out as an ether solution.

The microcolumn reactor can be utilized in the manner of a chip-cartridge also for rendering available almost any grignards and other metal-organic compounds for micro-fluid-syntheses in the form of a chip. The system is particularly advantageous since the solutions can easily be kept anhydrous and oxygen-free (for example, in contrast to microtiter plates and nanotiter plates) and, due to the small reaction volume, the emitted heat of

reaction can easily be dissipated. Thus an otherwise dangerous overheating of the reactor cannot occur.

According to the various possible applications described in the initial part of the specification, the proposed microcolumn reactor can be utilized with advantage in many a way.

All features disclosed in the specification, in the subsequent claims, and in the drawing can be substantial for the invention both, individually and in any combination with one another.

List of reference numerals

1	-	first substrate wafer
2	-	second substrate wafer
3	-	channel
41, 42	-	passage openings
411, 421	-	channel sections (in the second substrate wafer)
5	-	partially permeable sieve-like membrane
50	-	polymeric foil
51	-	transmission area (pores)
61, 62	-	openings (in the first substrate wafer)
7	-	micro-beads and/or cells
8	-	components such as, for example, optical detectors, analysis units, calorimeters, electrochemical detectors etc.
91, 92	-	valves
a, a1	-	section between passage openings
b	-	equidistant spaces between associated passage openings
c	-	different distances between associated passage openings
i	-	inlet for the mobile phase
o	-	outlet for the mobile phase
n	-	surface normal
p	-	fluidic pressure
A1 to D4	-	microcolumn reactors

CLAIMS

1. Microcolumn reactor for carrying out reactions on solid phases and/or biological cells comprising at least a first and a second substrate wafer (1; 2) being engaged to one another in a common plane, whereby at least one longitudinally extending channel (3) is inserted into at least one of said substrate wafers (1; 2), said channel, in a preselectable section (a) of its length, being captured by two openings (41, 42), which are passed through the substrate wafer, whereby filter elements are provided, characterized in that the passage openings (41, 42) are separated from the channel (3) by a partially permeable sieve-like membrane (5; 50), the transmission areas (51) of said membrane are so dimensioned that they preselectably prevent micro-beads and/or cells (7), which are introduced into the channel (3), from entering into the passage openings (41, 42), and in that the channel (3) is provided with at least two further openings (61, 62) outside of the section (a) captured by said passage openings (41, 42), said at least two further openings (61, 62) being adapted to enable a loading and/or a displacement of the micro-beads and/or cells (7), provided above the section (a), by applying a fluidic pressure (p), and in that means (91, 92) are provided for temporarily closing of at least one of the passage openings (41, 42) and of one of the openings (61, 62).

2. A microcolumn reactor as claimed in claim 1, characterized in that glass is selected for the first substrate wafer (1) and a silicon wafer for the second substrate wafer (2), whereby the channel (3) is inserted into the glass plate (1) and the surface of the silicon wafer (2) opposing said glass plate is entirely covered by a coat, into which a micro-structurized perforation is provided at least in the section of the passage openings (41, 42), said micro-structurized perforation being for forming transmission areas (51).

3. A microcolumn reactor as claimed in claim 1, characterized in that a glass plate and/or a plate made of synthetic material is/are selected for the first and/or for the second substrate wafer (1, 2), whereby the channel (3) is inserted into the first substrate wafer (1) and the surface of the second substrate wafer (2) opposing said first substrate wafer (1) is entirely covered by a membrane, into which a micro-structurized perforation is provided at least in the section of the passage openings (41, 42), said micro-structurized perforation being for forming transmission areas (51).

4. A microcolumn reactor as claimed in claim 3, characterized in that the membrane is formed by a perforated polymeric foil (50) covering the second substrate wafer (2).

5. A microcolumn reactor as claimed in claim 2, characterized in that the first and the second substrate wafer (1, 2) are connected to one another by anodic bonding.

6. A microcolumn reactor as claimed in claim 2 or 3, characterized in that the first and the second substrate wafer (1, 2) are connected to one another by adhesives outside of the range of the channel (3).

7. A microcolumn reactor as claimed in claim 3, characterized in that the first and the second substrate wafer (1, 2) are connected to one another by externally provided clamping means.

8. A microcolumn reactor as claimed in claims 1, 2 or 3, characterized in that the passage openings (41, 42) are connected to a respective channel (411, 421) which is arranged in the plane of the second substrate wafer (2) and extends to the rim portion of the substrate.

9. A microcolumn reactor as claimed in one of the preceding claims, characterized in that a channel (3) is defined by a plurality of passage openings (41, 42), whereby respectively correlated passage openings, which constitute an inlet (i) and an outlet (o), and passage openings, which define a section (a) of the channel (3), are arranged to one another equidistantly (b) or in variable and different distances (c).

10. A microcolumn reactor as claimed in claim 9, characterized in that, when a plurality of correlated passage openings (41, 42) are provided on a common substrate or when a plurality of discrete microcolumn reactors are fluidically interconnected, the respective distances (a; a1) of correlated passage openings, which form one inlet and one outlet, are designed, adapted to the actual reaction process, of different length.

11. A microcolumn reactor as claimed in claim 1, characterized in that a plurality of substrate wafers, which have a channel (3) and two passage openings (41, 42) each, are linearly and/or in a plurality of planes fluidically interconnected with one another as separate units each, whereby further components (8) such as, for example, optical detectors, analysis units, calorimeters, electrochemical detectors etc. are provided at preselectable connection sites.

12. A microcolumn reactor as claimed in claim 1, characterized in that a plurality of substrate wafers, which have a channel (3) and two passage openings (41, 42) each, are linearly and/or in a plurality of planes fluidically interconnected with one another as separate units each, whereby further micro-structurized components (8) such as, for example, optical detectors, analysis units, calorimeters, electrochemical detectors etc. are provided integrated in the entire system.

13. A microcolumn reactor as claimed in claims 1 and 2, characterized in that the passage openings (41, 42) are, in parallel to the surface normal n, designed by two channel ranges in the shape of two truncated pyramids standing via their small base faces top-to-top one upon the other, whereby an Si(100)-wafer is used for the substrate wafer 2 which on both of its sides is provided with an etching mask, the first masking face of which is provided with transmission areas (51) at least across the passage openings (41, 42), and whereby the second masking face disposed on the opposing wafer face is provided with recesses, the openings of which correspond to the smallest inside cross section of the passage openings (41, 42).

14. A microcolumn reactor as claimed in claims 1, 2, and 8, characterized in that an Si-wafer of 100-orientation or 110-orientation is used for the second substrate wafer, which on one of its sides is provided with a sieve pore membrane mask structure (5) which, in the range of the further extending channel 411, 412, is accompanied by a window corresponding to the channel width, which extends up to the rim of the chip, and the opposite side of the Si-wafer is entirely covered by a protective coat, for example Si_3N_4, which is etching resistant.

15. A microcolumn reactor as claimed in claim 1, characterized in that the membrane (5) is formed by a nano-porous thin-layer membrane, the pore sizes of which are defined in a range between 5...500 nm.

ABSTRACT

The invention relates to a microcolumn reactor for carrying out reactions on solid phases and/or with biological cells. The aim of the invention is to provide a microcolumn reactor for processes of synthesis and separation on small sample volumina that replaces an alternating sequence of binding and elution processes on a phase that is stationary bound during a test series and makes it possible to exchange the stationary phase, for example once it has been loaded. To this end, the microcolumn reactor consists of at least one first and one second substrate wafer (1; 2) that are linked with one another across their surfaces. At least one of the substrate wafers (1; 2) is provided with an elongate channel (3) which comprises in a defined section (a) of its length two passage openings (41, 42) that are implemented in the opposite substrate wafer. Said openings (41, 42) are separated from the channel (3) by a partially permeable sieve-type membrane (5). The areas of acceptance (51) of said membrane have a diameter that is chosen in such a manner as to specifically prevent microbeads and/or cells introduced into the channel from entering the passage openings (41, 42). The channel (3) is provided with at least two further openings (61, 62) outside the section (a) that is comprised by the passage openings (41, 42). Said further openings permit the microbeads and/or cells that are provided above the section (a) to be introduced and/or displaced by applying a fluid pressure (p). Means are provided that temporarily close at least one of the passage openings (41, 42) and one of openings (61, 62).

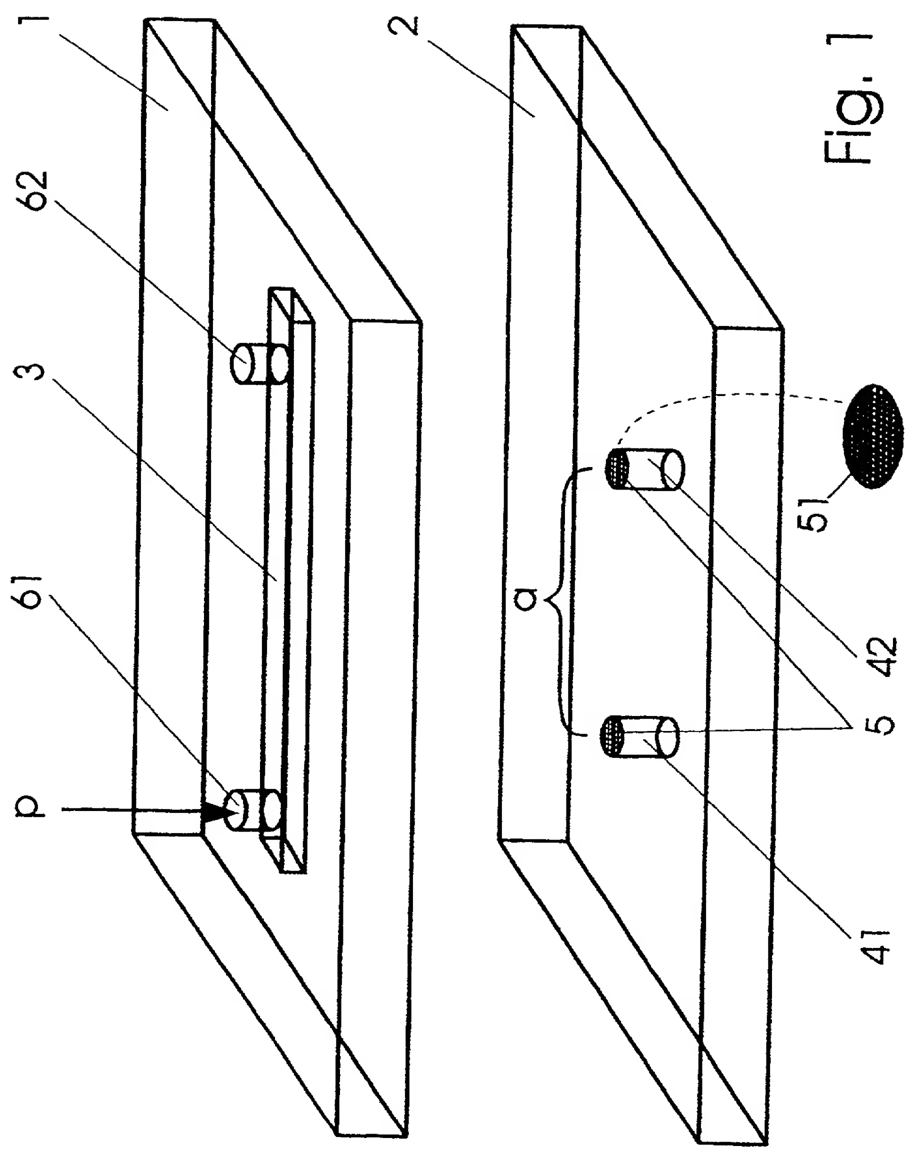

Fig. 1

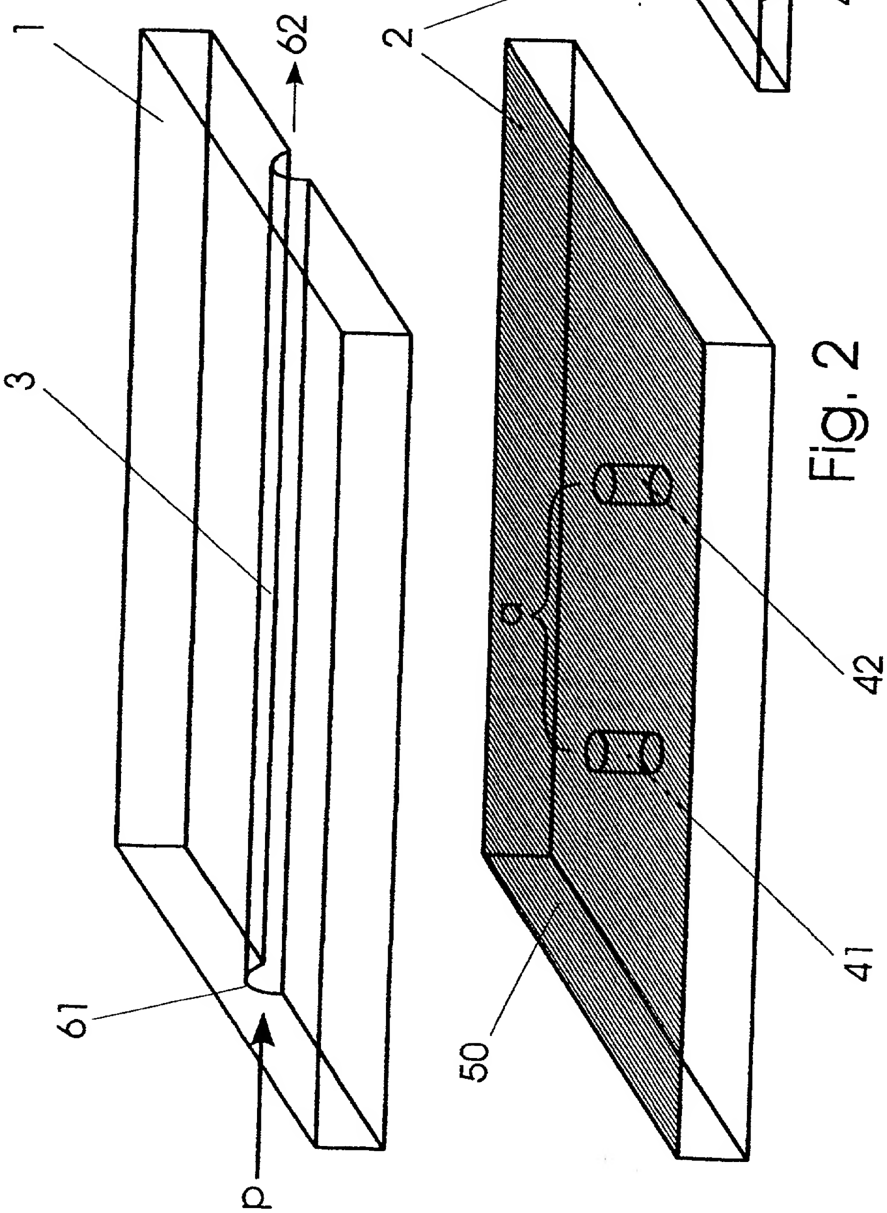

Fig. 2

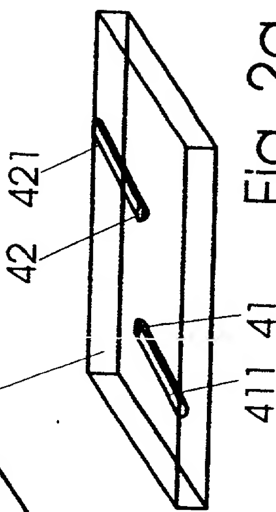

Fig. 2a

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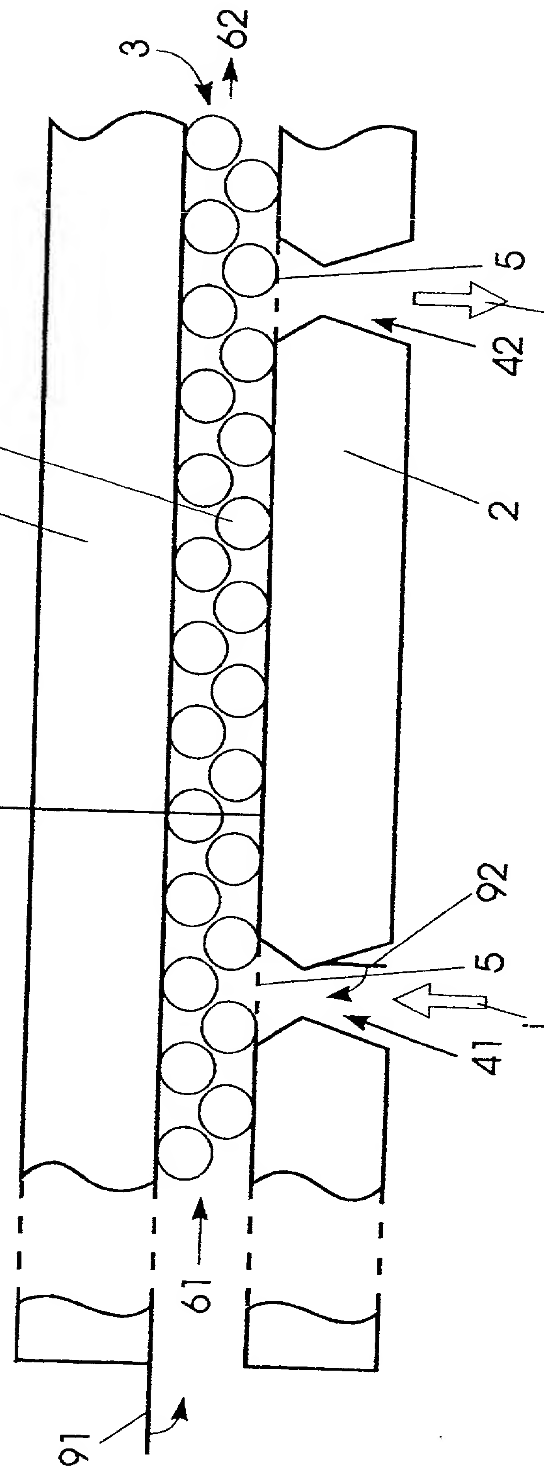

Fig.3

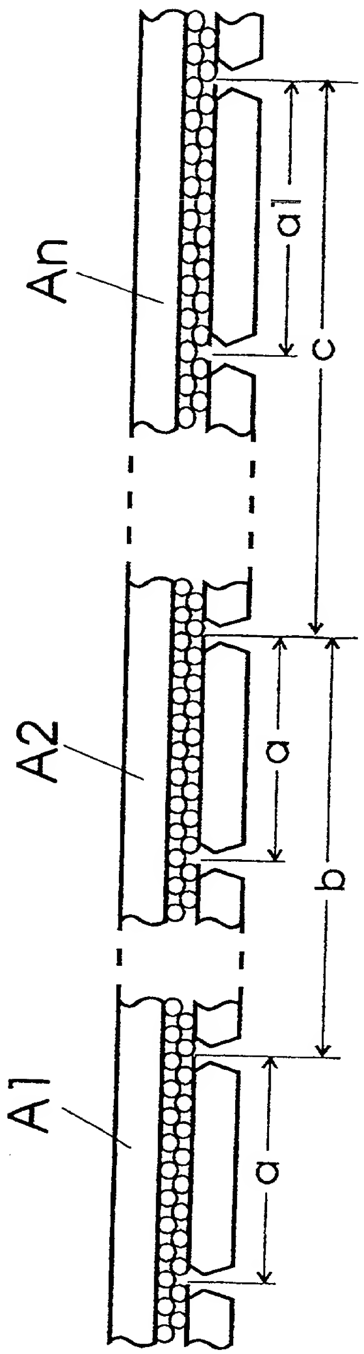

Fig. 4

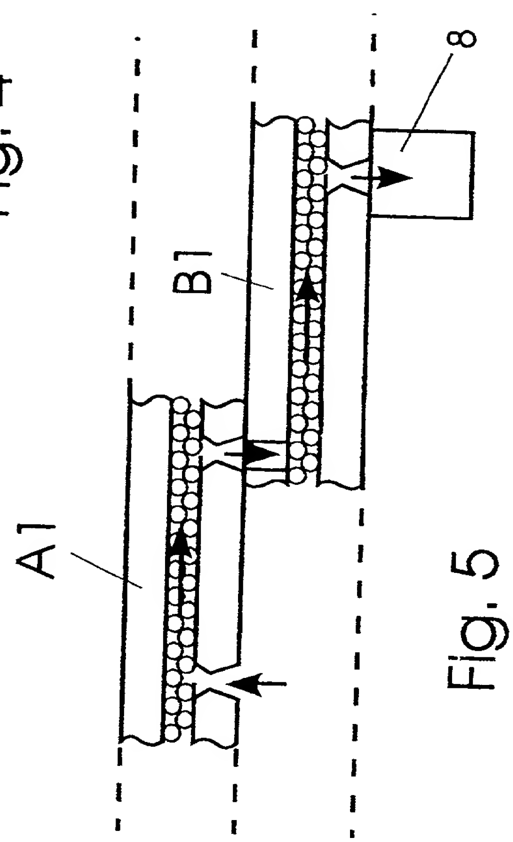

Fig. 5

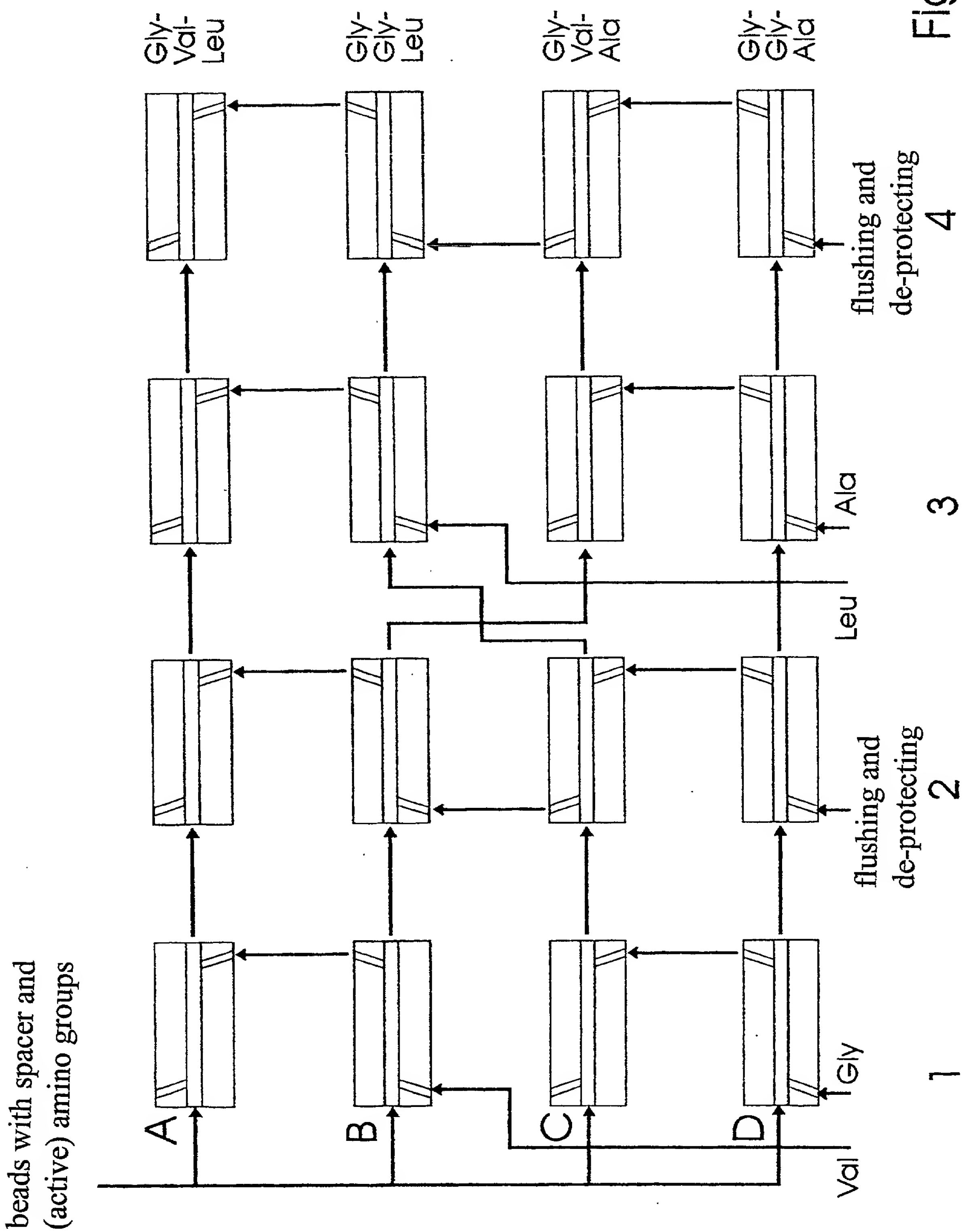
beads with spacer and (active) amino groups
A
B
C
D
Val
Gly
Leu
Ala
flushing and de-protecting
flushing and de-protecting
1
2
3
4
Gly-Val-Leu
Gly-Gly-Leu
Gly-Val-Ala
Gly-Gly-Ala

Fig. 6

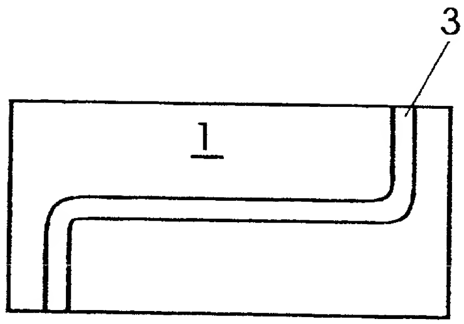

Fig. 7a

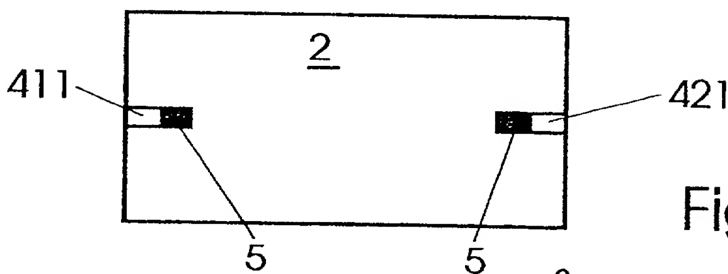

Fig. 7b

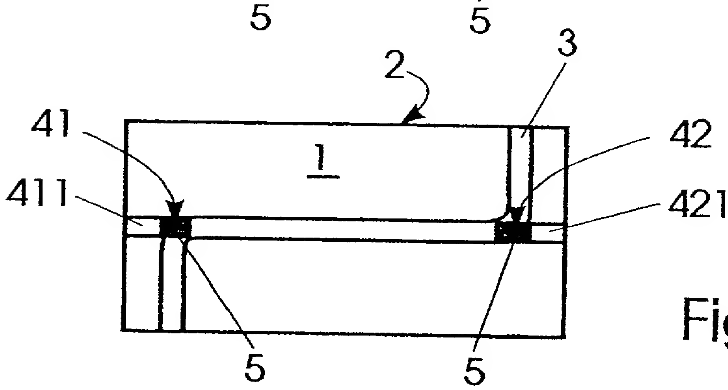

Fig. 7d

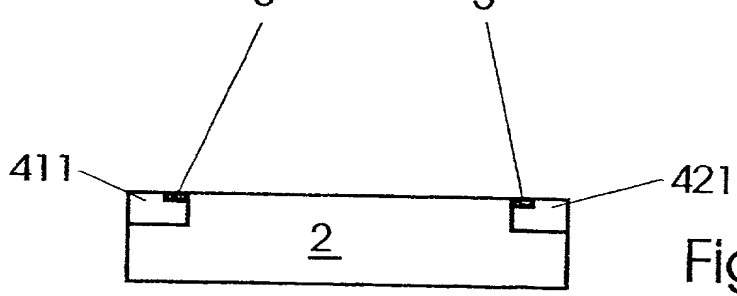

Fig. 7c

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Continued) (Includes Reference to PCT International Applications)	Attorney's Docket Number

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:					
U.S. APPLICATIONS			STATUS (Check One)		
U.S. Application Number	U. S. Filing Date		Patented	Pending	Abandoned
PCT APPLICATIONS DESIGNATING THE U.S.					
PCT Application No.	PCT Filing Date	U.S. Serial Numbers Assigned (if any)			

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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C. Bruce Hamburg	Reg. No. 22,389	Marvin Turken	Reg. No. 18,330
Lainie E. Dolinger	Reg. No. 36,123	Alfred D'Andrea	Reg. No. 27,752

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Full Name of Sole or First Inventor	Inventor's Signature	Date
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Full Name of Second Joint Inventor, if any	Inventor's Signature	Date
Residence		Citizenship
Post Office Address		

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to PCT International Applications)	Attorney's Docket Number

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

MICROCOLUMN REACTOR

the specification of which (check only one item below):

[ ] is attached hereto.

[ ] was filed as United States application
Serial No. ______
on ______,
and was amended
on ______ (if applicable).

[X] was filed as PCT international application
Number PCT/EP00/01701,
on February 25, 2000
and was amended under PCT Article 19
on ______ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

Country (if PCT indicate "PCT")	Application Number	Date of Filing (day, month, year)	Priority Claimed Under 35 USC 119
DE	199 10 392.5	05.03.1999	[X] Yes [ ] No
			[ ] Yes [ ] No
			[ ] Yes [ ] No
			[ ] Yes [ ] No
			[ ] Yes [ ] No